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Growth and physiological responses of cotton (*Gossypium hirsutum* L.) to elevated carbon dioxide and ultraviolet-B radiation under controlled environmental conditions

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ABSTRACT

Better understanding of crop responses to projected changes in climate is an important requirement. An experiment was conducted in sunlit, controlled environment chambers known as soil–plant–atmosphere–research units to determine the interactive effects of atmospheric carbon dioxide concentration [CO_2] and ultraviolet-B (UV-B) radiation on cotton (*Gossypium hirsutum* L.) growth, development and leaf photosynthetic characteristics. Six treatments were used, comprising two levels of [CO_2] (360 and 720 μmol mol^{-1}) and three levels of 0 (control), 7.7 and 15.1 kJ m^{-2} d^{-1} biologically effective UV-B radiations within each CO_2 level. Treatments were imposed for 66 d from emergence until 3 weeks after the first flower stage. Plants grown in elevated [CO_2] had greater leaf area and higher leaf photosynthesis, non-structural carbohydrates, and total biomass than plants in ambient [CO_2]. Neither dry matter partitioning among plant organs nor pigment concentrations was affected by elevated [CO_2]. On the other hand, high UV-B (15.1 kJ m^{-2} d^{-1}) radiation treatment altered growth resulting in shorter stem and branch lengths and smaller leaf area. Shorter plants at high UV-B radiation were related to internode lengths rather than the number of mainstem nodes. Fruit dry matter accumulation was most sensitive to UV-B radiation due to fruit abscission. Even under 7.7 kJ m^{-2} d^{-1} of UV-B radiation, fruit dry weight was significantly lower than the control although total biomass and leaf photosynthesis did not differ from the control. The UV-B radiation of 15.1 kJ m^{-2} d^{-1} reduced both total (43%) and fruit (88%) dry weights due to smaller leaf area and lower leaf net photosynthesis. Elevated [CO_2] did not ameliorate the adverse effects of UV-B radiation on cotton growth and physiology, particularly the boll retention under UV-B stress.

Key-words: *Gossypium hirsutum* L.; $CO_2 \times$ UV-B interactive effects; dry matter production and partitioning; non-structural carbohydrates; pigments; photosynthesis.

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INTRODUCTION

Continued depletion of the stratospheric ozone layer, mainly due to recent increases in atmospheric chlorofluorocarbons (CFCs), methane and nitrous oxide, is of concern because the ozone column is the primary attenuator of solar UV-B radiation (280–320 nm). Reductions in the ozone column have led to substantial increases in UV-B radiation at the surface of the Earth with the amount dependent on atmospheric and geographic factors (WMO 1995; Madronich *et al*. 1998). Intensified current and projected UV-B radiation is known to alter and affect plant growth, development and physiological processes (Dai *et al*. 1992; Gonzalez *et al*. 1996; Nogues *et al*. 1999). Damage due to UV-B radiation results in inhibition of photosynthesis, degradation of protein and DNA, and increased oxidative stress (Jordan, Chow & Anderson 1992; Stapleton 1992; Sullivan 1997). In general, enhanced UV-B radiation affects plant growth and/or yield of most crops (Krupa, Kickert & Jäger 1998). The sensitivity of plants to UV-B radiation, however, varies among species, and is influenced by environmental factors, such as water regime, photosynthetically active radiation (PAR) and nutrient status (Murali & Teramura 1985; Balakumar, Hani & Paliwal 1993; Mark & Tevini 1996). As in other crops, enhanced UV-B radiation may alter cotton growth and development. Song *et al*. (1999) found that enhanced UV radiation reduced growth and yield in field-grown cotton.

Along with increase in UV-B radiation, climate projections indicate changes in other major environmental factors such as the increase in atmospheric carbon dioxide concentration [CO_2], temperature, and variability in precipitation (Houghton *et al*. 2001). The [CO_2] in the atmosphere has increased by more than 28% since the beginning of the Industrial Revolution mainly because of the burning of fossil fuels and deforestation. The most recent future scenarios of greenhouse gases in the atmosphere indicate that [CO_2] could increase from current levels of approximately 360 μmol mol^{-1} to between 540 and 970 μmol mol^{-1} by the end of the twenty-first century (Houghton *et al*. 2001). Of the environmental variables, CO_2 is an important greenhouse gas and its increase in the atmosphere is known to increase C_3-type crop yields (Kimball 1983). Elevated

atmospheric [CO_2] generally enhances leaf and canopy CO_2 assimilation rates because CO_2 is not only the substrate for photosynthesis but also a competitive inhibitor of oxygenation of RuBP carboxylase and reduces photorespiration (Lawlor & Mitchell 2000).

The response of crop plants to UV-B radiation in the presence of other environmental stress factors is not clear and has not received much attention. Studies conducted so far indicate that effects of UV-B radiation with other environmental variables such as enhanced [CO_2] (Teramura, Sullivan & Ziska 1990; Rozema *et al*. 1997; Visser *et al*. 1997), high temperature (Mark & Tevini 1996), water deficit (Nogues *et al*. 1998; Alexieva *et al*. 2001) are both additive and interactive. Few studies have evaluated the effects of UV-B radiation on the growth and development of cotton, an important agronomic crop grown in a wide range of environmental conditions spanning latitudes 40°N and 40°S, although the influences of other environmental factors such as atmospheric [CO_2], temperature, water and nutrients, on plant growth and development have been well investigated in cotton (Christiansen 1986; Gerik, Oosterhuis & Torbet 1998; Reddy, Hodges & Kimball 2000).

Reddy *et al*. (2000) reported that canopy photosynthesis of cotton increased by 40% when [CO_2] was increased from 320 to 720 μmol mol^{-1} for plants grown at 20 and 32 °C, and increased by about 80% when plants were grown under optimum temperature (26–28 °C) conditions. Kimball & Mauney (1993) found that cotton grown under 550 μmol CO_2 mol^{-1} had a 35% higher biomass, 40% higher fruit weight and 60% higher lint yield than plants grown under 350 μmol CO_2 mol^{-1} under free-air CO_2 enrichment studies. However, there is a significant knowledge gap on the interactive effects of UV-B radiation and [CO_2] on plants. We hypothesize that the CO_2 enhancement effect may be reduced by concurrent exposure of plants to elevated UV-B radiation. The objective of this study was thus to determine the interactive effects of elevated [CO_2] and UV-B radiation on cotton growth, development, and physiology under controlled environment conditions. Information related to whole plant and leaf-level processes will contribute to filling gaps in our knowledge base and understanding.

MATERIALS AND METHODS

Soil–plant–atmosphere research units

The experiment was conducted at the Agriculture and Forestry Experiment Station of Mississippi State (33°28′ N, 88°47′ W), Mississippi, USA in 2001 using six soil–plant–atmosphere research (SPAR) units. The SPAR facility has the capability to precisely control temperature and [CO_2] at predetermined set points for plant growth studies under near ambient levels of PAR. Details of the SPAR units have been described by Reddy *et al*. (2001). Each SPAR unit consists of a steel soil bin (1 m deep by 2 m long by 0.5 m wide), and a Plexiglas chamber (2.5 m tall by 2 m long by 1.5 m wide) to accommodate aerial plant parts, a heating and cooling system, and an environment monitoring and control system. The Plexiglas chamber is completely opaque to solar UV radiation, but transmits more than 95% of incoming PAR (wavelength 400–700 nm) (Table 1). During the experiment, the incoming solar radiation (285–2800 nm) outside the SPAR units was measured with a pyranometer (Model 4–48; The Eppley Laboratory Inc., Newport, RI, USA). The average daily total solar radiation during the experiment was 18 ± 4.9 MJ m^{-2} d^{-1}. The air temperature and [CO_2] in each SPAR unit were monitored and adjusted every 10 s throughout the experiment.

The dew point temperatures were monitored with gold mirror hygrometers installed inside the return airline, and were also collected at 10 s intervals and summarized over 900 s intervals. The data were separated into day and night periods. The daytime vapour pressure deficit (VPD) of each treatment was calculated based on air temperature and dew point temperature. The PAR transmittance of all chambers were determined on two typical clear days between 1000 and 1400 h using seven 1 m line quantum sensors (Li-Cor Inc., Lincoln, NE, USA). Daily mean temperatures, average daytime VPD, and PAR transmittances of all treatments are presented in Table 1.

Table 1. Measured [CO_2] and Plexiglas transmittance of photosynthetically active radiation (PAR) from a typical day, daily mean UV-B radiation dosage, mean temperature (T), and daytime vapour pressure deficit (VPD) during the experimental period for each treatment

Treatment						
CO_2 (μmol mol^{-1})	UV-B (kJ m^{-2} d^{-1})	CO_2 (μmol mol^{-1})	Mean UV-B (kJ m^{-2} d^{-1})	Mean T (°C)	VPD (kPa)	Transmittance of PAR (%)
360	0.0	359.7 ± 0.7	0.00 ± 0.00	26.58 ± 0.04	1.68 ± 0.28	96.2 ± 0.6
	7.7	361.3 ± 1.8	7.63 ± 0.05	26.59 ± 0.03	1.96 ± 0.15	96.3 ± 0.5
	15.1	360.1 ± 0.7	15.03 ± 0.08	26.45 ± 0.09	1.59 ± 0.08	97.3 ± 0.5
720	0.0	723.1 ± 1.0	0.00 ± 0.00	26.59 ± 0.03	1.64 ± 0.20	98.6 ± 0.2
	7.7	725.0 ± 1.4	7.74 ± 0.04	26.59 ± 0.03	1.89 ± 0.21	95.7 ± 0.5
	15.1	723.3 ± 1.0	15.12 ± 0.09	26.50 ± 0.05	1.79 ± 0.19	96.0 ± 0.5

Each value represents the mean ± SE of data of 40 values from a day for [CO_2] and PAR transmittance, or 65 d from 6 August to 10 October 2001 for UV-B radiation, VPD and temperature.

Plant culture

Seeds of cotton, cv. NuCOTN 33B, were sown on 1 August 2001 in fine sand of the SPAR soil bins in 11 rows of five plants per row. Emergence date was 5 d later. The temperatures in all units were maintained at 30/22 °C (day/night) during the experiment. Plants were watered three times a day with half-strength Hoagland's nutrient solution delivered at 0800, 1200 and 1700 h to ensure favourable nutrient and water conditions for plant growth. Irrigation was provided through an automated and computer-controlled drip system. Variable-density black shade cloths around the edges of plants were adjusted regularly to match plant height in order to simulate natural shading in the presence of other plants.

Treatments

The six treatments included two CO_2 levels of 360 μmol mol^{-1} (ambient) and 720 μmol mol^{-1} (elevated), and three daily biologically effective UV-B radiation intensities of 0 (control), 7.7 and 15.1 kJ m^{-2} within each CO_2 level. The [CO_2] and UV-B radiation treatments were imposed from emergence. The UV-B doses of 7.7 and 15.1 kJ m^{-2} d^{-1} are designed to simulate the maximum level of UV-B that would be received on a clear day on the summer solstice with either ambient levels of ozone or with a 30% ozone depletion in Mississippi's cotton production region, as calculated by an empirical model (Green, Cross & Smith 1980).

Square-wave UV-B supplementation systems were used to provide respective UV-B radiation in this study under near ambient PAR. The UV-B radiation was delivered to plants for 8 h, each day, from 0800 to 1600 h by eight fluorescent UV-313 lamps (Q-Panel Company, Cleveland, OH, USA) driven by 40 W dimming ballasts. The lamps were wrapped with pre-solarized 0.08 mm cellulose diacetate film to filter UV-C (< 280 nm) radiation. The cellulose diacetate film was changed at 3–4 d intervals. The amount of UV-B energy delivered at the top of the plant canopy was checked daily at 1000 hours with a UVX digital radiometer (UVP Inc., San Gabriel, CA, USA) and calibrated against an Optronic Laboratory (Orlando, FL, USA) Model 754 Spectroradiometer, which was used to initially quantify lamp output. The lamp power was adjusted, as needed, to maintain the appropriate UV-B radiation level in each treatment and to maintain the distance from lamps to the top of plants at about 0.5 m throughout the experiment. The mean UV-B dosages measured at three different locations in each SPAR unit corresponding to the permanent rows for all treatments during the experimental period are presented in Table 1. After averaging the radiation dosages for the plants within UV-B treatments, the actual dosages for the two levels of UV-B treatments were 7.7 and 15.1 kJ m^{-2} d^{-1}, respectively. Square-wave UV-B supplemental systems in controlled environments usually provide higher UV-B radiation and disproportionate spectral conditions compared to ambient condition (Musil *et al*. 2002), and there is no UV-B supplied in the control treatment. This is likely to exaggerate the UV-B response in plants, and thus caution will be necessary in extrapolating the findings from this study to the field.

Measurements

Plant height and the number of mainstem nodes were determined from nine plants (three centre plants in each row) at 3 d intervals from 10 to 66 d after emergence (DAE). These plants were also used to record dates of appearance of the first floral bud of 3 mm in length (first square, FS) and first flower (FF), and durations in days from emergence to FS and from FS to FF. The plants were harvested at the fourth true leaf stage (14 DAE), early floral bud stage (29 DAE) and finally at 3 weeks after the first flower stage (66 DAE). Plants in the odd rows of each SPAR unit were harvested at 14 DAE. The five rows after the first sampling were spaced 33 cm apart. The two even rows were harvested 29 DAE, and the remaining three rows (with a 67 cm row space) were finally harvested 66 DAE. Plants were separated into leaves and stems at the first two harvests, and the leaf area and dry weights of leaves and stems were determined. At the final harvest, plant height, fruiting branch lengths, numbers of mainstem nodes, fruiting branches, fruiting sites, squares and bolls on each plant were recorded. The rooting medium was removed and roots washed over a fine screen, dried and weighed. Mean internode length was calculated by dividing plant height by the number of mainstem nodes. Fruit (squares + bolls) abscission was calculated as the difference between the number of fruiting sites and the number of fruits.

Net photosynthetic rates (P_n) of the uppermost fully expanded mainstem leaf from five plants in each treatment were measured between 1000 and 1200 h using a LI-6400 portable photosynthesis system (Li-Cor Inc.) at 28, 44 and 64 DAE. When measuring leaf P_n, the PAR, provided by a 6400–02 LED light source, was set to 1500 μmol m^{-2} s^{-1}. The temperature in the leaf cuvette was set to 30 °C, and [CO_2] matched CO_2 treatments.

Chlorophyll *a*, chlorophyll *b*, carotenoid and non-structural carbohydrate (including glucose, fructose, sucrose, and starch) concentrations of the leaves at the same position were also determined at 45 and 65 DAE. For chlorophyll measurement, five leaf discs (0.385 cm^2 each) were punched from a leaf and placed in a vial with 4 mL of dimethyl sulphoxide. Three samples were collected from each treatment, and incubated at room temperature for 24 h, in the dark, to allow for complete extraction of chlorophyll into the solution. The absorbance of the extract was measured using a Pharmacia UltraSpec Pro UV/VIS spectrophotometer (Pharmacia, Cambridge, UK) at 470, 648 and 664 nm and used to calculate chlorophyll *a*, chlorophyll *b*, and carotenoid concentrations (Chappelle, Kim & McMurtrey 1992).

Five leaf discs from each leaf were collected at 0830 h using the same method described above and placed in a test tube with 2 mL of 80% (v/v) cold ethanol. Three leaf sam-

ples were obtained from each treatment and immediately stored at –80 °C to determine leaf non-structural carbohydrates. The modified method of Hendrix (1993) was used to extract and quantify the concentrations of leaf non-structural carbohydrates. Briefly, soluble sugars were extracted by incubating leaf tissue with 2 mL of the ethanol, in an 80 °C water bath, for 15 min. The extraction was repeated three times and the supernatants were combined and brought to a 6 mL volume with 80% ethanol. Sixty milligrams of finely ground activated charcoal was added to each sample tube, covered and shaken by hand. After 5 min, the tubes were centrifuged at 3000 × *g* for 15 min to obtain a clear extract. Four 40 μL aliquots from each sample were pipetted into separate wells of a microtitration plate and dried at 50 °C for 30 min to remove the ethanol. Eight wells in the first column of each microtitration plate were filled with 20 μL glucose standard solutions of 0, 5, 13, 25, 50, 125, 250 and 500 mg L^{-1}, respectively. To each sample well, 20 μL deionized water was added, and then 100 μL glucose-6P dehydrogenase/iodonitrotetrazolium violet (INT) mixture (glucose kit 115 A; Sigma Chemical Company, St Louis, MO, USA) was added under reduced room illumination. Plates were incubated at 37 °C for 15 min and the absorbance measured at 490 nm with an Emax precision microplate reader (Emax, Sunnyvale, CA, USA). A glucose standard curve was obtained for each plate based on the standard concentrations and absorbance. Sample glucose concentration was calculated according to the standard curve, absorbance, extract volume, and leaf disc area. Subsequently, 10 μL phosphoglucose isomerase (PGI enzyme, 0.25 units) and 10 μL invertase (83 units) were added to each well separately, re-incubated at 37 °C for 15 min each time. The absorbance was determined at 490 nm to obtain concentrations of fructose and sucrose.

After extraction of the soluble sugars, 1 mL of 0.1 M KOH was added to the test tube containing the tissue residue, and the tubes placed for 1 h in a boiling waterbath. An α-amylase and amyloglucosidase preparation was used to hydrolyse starch to glucose as described by Hendrix (1993). After centrifuging the hydrolysate, the supernatant was collected and the glucose concentration determined as above. Starch concentration in the sample was calculated according to the glucose concentration multiplied by 0.9 to account for water loss when glucose units are linked. The sum of glucose, fructose, sucrose and starch was defined as total non-structural carbohydrate.

Data analysis

A uniformity test of the SPAR units conducted in a previous study by determining sorghum growth parameters under the same controlled environments indicated no statistical differences among all SPAR units (Reddy, personal communication, 2000). Therefore, six treatments were randomly arranged in six identical SPAR units. Except for the two treatment factors of $[CO_2]$ and UV-B radiation, the other growth conditions were the same in all treatments (Table 1). All growth measurements were made on nine plants and physiological measurements were made on either three or five plants for each treatment. Linear and sigmoid regressions were performed for the number of mainstem nodes and plant height, respectively, against days after emergence. Data were analysed by the analysis of variance (ANOVA) procedures in SAS (SAS Institute Inc. 1997) to determine the main and interactive effects of $[CO_2]$ and UV-B radiation on growth, dry matter accumulation, leaf P_n, and concentrations of leaf pigments and non-structural carbohydrates. If the hypothesis of equal means was rejected by the ANOVA test, Fisher LSD procedures at $P = 0.05$ probability level (SAS Institute Inc. 1997) were employed to distinguish differences among treatment means for the growth and physiological processes measured.

RESULTS

Plant growth and development

Plants grown under 720 μmol CO_2 mol^{-1} were typically 25–50 cm taller than those grown in 360 μmol CO_2 mol^{-1}, depending on UV-B exposure (Fig. 1). Changes in plant height from emergence up to 3 weeks after first flowering followed a sigmoid growth pattern. The maximum mainstem elongation rate was achieved between 35 and 45 DAE for all treatments, but the elongation rates differed substantially among the treatments. Plants irradiated with 15.1 kJ m^{-2} d^{-1} UV-B radiation had significantly lower stem elongation rates in both CO_2 levels, compared to plants in either the 0 or 7.7 kJ m^{-2} d^{-1} UV-B treatments. At the final harvest (66 DAE), plants grown in 720 μmol CO_2 mol^{-1} were 22% taller than plants grown under 360 μmol CO_2 mol^{-1}, averaged across the UV-B treatments. Plants irradiated with UV-B radiation were significantly shorter than the controls (except for 7.7 kJ UV-B treatment at 360 μmol CO_2 mol^{-1}).

Unlike plant height, mainstem nodes increased linearly ($R^2 = 0.99$) as plants aged in all treatments (data not shown). The node appearance rates (slopes of the lines) did not differ among the UV-B treatments, but elevated $[CO_2]$ caused a slight increase in node addition rate ($Y = 0.33X - 1.28$ for 360 μmol CO_2 mol^{-1} and $Y = 0.36X - 1.61$ for 720 μmol CO_2 mol^{-1}, where Y is the number of mainstem nodes, and X is days after emergence). Averaged across the UV-B treatments, it took about 3.0 d (slope = 0.33) to produce a leaf (or node) on the main stem under ambient $[CO_2]$ and about 2.8 d (slope = 0.36) under elevated $[CO_2]$. At the final harvest, mean internode lengths of the 0, 7.7 and 15.1 kJ UV-B-treated plants were 8.5, 7.9 and 4.5 cm, respectively, under ambient $[CO_2]$; and 9.4, 8.3 and 5.4 cm, respectively, under elevated $[CO_2]$.

No CO_2 × UV-B radiation interaction effect was detected for leaf area development (Table 2). Plants grown in elevated $[CO_2]$ had significantly greater leaf area than plants in ambient $[CO_2]$ at 14 DAE. Leaf area was reduced by UV-B treatments of 7.7 and 15.1 kJ m^{-2} d^{-1} at 24 DAE. At 66 DAE, only plants exposed to 15.1 kJ UV-B had significantly

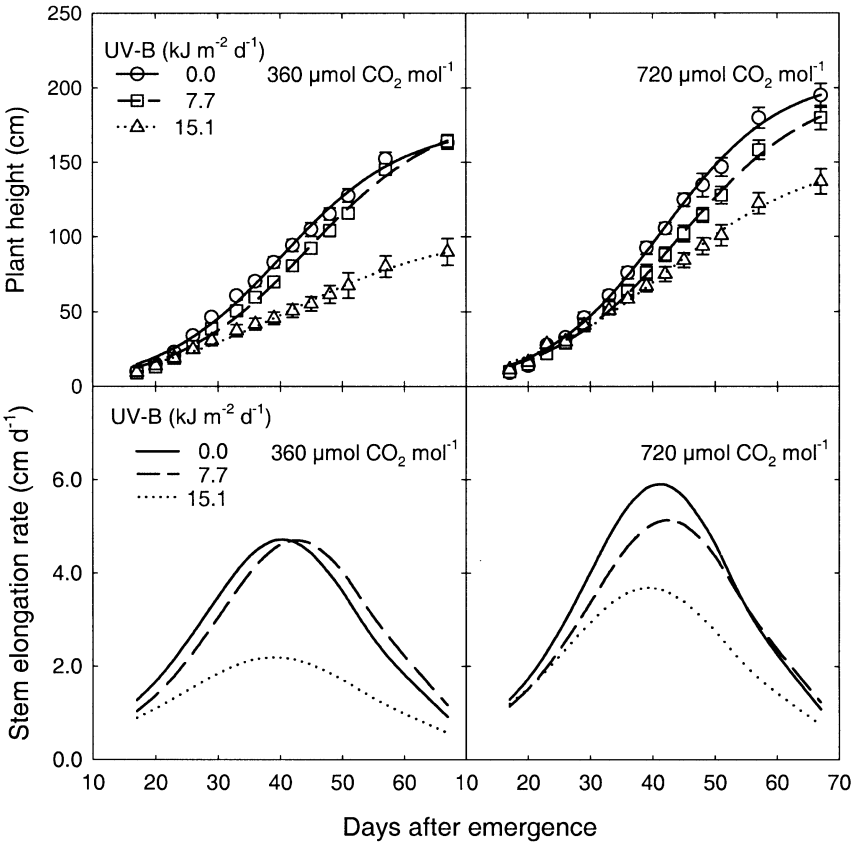

Figure 1. Changes in plant height and stem elongation rate of cotton plants grown under 360 and 720 μmol CO_2 mol^{-1} as affected by UV-B radiation. Each data point represents the mean ± SE of nine plants. The lines are regressions fitted to the data using a sigmoid equation for plant height and a first derivative of the sigmoid equation for stem elongation rate.

smaller leaf area (47–50%) than plants in other treatments of CO_2 and UV-B radiation.

Neither elevated [CO_2] nor UV-B radiation affected the dates of appearance of the first floral buds and flowers (data not shown). A significant $CO_2 \times$ UV-B interactive effect was detected for the number of fruiting branches, branch lengths and fruit abscission (Table 3). Elevated [CO_2] significantly increased the numbers of fruiting branches, fruiting sites and squares per plant and mean branch length, but did not affect the number of bolls retained per plant. In contrast to [CO_2], UV-B radiation significantly decreased most reproductive growth parameters, except for the number of fruiting branches. Fruit abscission in UV-B-treated plants was significantly higher than in control plants in both ambient and elevated CO_2 levels. Floral bud abscission was more than young boll abscission when plants were grown under UV-B radiation (data not shown).

Dry matter accumulation and partitioning

Elevated [CO_2] significantly increased dry matter accumulation in leaves and stems at all sampling dates (Tables 4 & 5). Dry weights of leaves and stems did not differ among the UV-B radiation treatments at 14 DAE, but at 24 DAE,

Table 2. Effects of elevated CO_2 and UV-B radiation on leaf area (cm^2 $plant^{-1}$) of cotton plant, 14, 24, and 66 d after emergence

CO_2 (μmol mol^{-1})	UV-B (kJ m^{-2} d^{-1})	Day after emergence		
		14	24	66
360	0.0	98 ± 6 ab*	589 ± 48 b	6111 ± 627 c
	7.7	87 ± 3 bc	471 ± 50 c	6776 ± 331 bc
	15.1	80 ± 6 c	455 ± 27 c	3064 ± 251 e
720	0.0	102 ± 6 a	721 ± 32 a	8456 ± 432 a
	7.7	104 ± 4 a	546 ± 42 bc	7738 ± 440 ab
	15.1	110 ± 7 a	588 ± 31 b	4502 ± 354 d
P (CO_2)		0.0002	0.0011	< 0.0001
P (UV-B)		0.5991	0.0009	< 0.0001
P ($CO_2 \times$ UV-B)		0.0702	0.7166	0.2611

Each value is the mean ± SE of nine plants.
*Means followed by the same letter within a column are not significantly different ($P > 0.05$).

Table 3. Effect of elevated CO_2 and UV-B radiation on fruiting branch length (cm branch^{-1}), the number of fruiting branches, fruiting sites, bolls, floral buds, and fruit abscission (no. plant^{-1}), 66 d after emergence

CO_2 (μmol mol^{-1})	UV-B (kJ m^{-2} d^{-1})	Fruiting branches	Branch length	Fruiting sites	Bolls	Floral buds	Fruit abscission
360	0.0	14.1 ± 0.4 c*	24.1 ± 1.5 b	47.3 ± 5.7 c	18.7 ± 2.4 ab	24.3 ± 3.6 c	4.3 ± 0.6 d
	7.7	15.8 ± 0.3 b	30.2 ± 0.8 a	55.8 ± 2.4 bc	14.7 ± 1.5 bc	34.1 ± 1.6 b	7.0 ± 0.8 c
	15.1	14.2 ± 0.6 c	11.9 ± 1.3 d	33.9 ± 3.4 d	5.2 ± 1.1 d	21.4 ± 3.1 c	7.2 ± 1.9 c
720	0.0	16.2 ± 0.5 b	31.2 ± 1.3 a	63.1 ± 6.9 ab	21.9 ± 2.9 a	34.4 ± 3.6 b	6.8 ± 0.9 cd
	7.7	16.3 ± 0.3 b	31.0 ± 1.5 a	67.4 ± 3.0 a	14.4 ± 1.6 c	41.2 ± 1.9 a	11.8 ± 0.9 b
	15.1	17.6 ± 0.5 a	19.5 ± 1.4 c	51.4 ± 3.4 c	3.8 ± 1.2 d	32.8 ± 1.9 b	14.9 ± 1.0 a
P (CO_2)		< 0.0001	< 0.0001	< 0.0001	0.6681	< 0.0001	< 0.0001
P (UV-B)		0.0944	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
P ($CO_2 \times$ UV-B)		0.0078	0.0240	0.6715	0.2686	0.6122	0.0156

Each value is the mean ± SE of nine plants.
*Means followed by the same letter within a column are not significantly different ($P > 0.05$).

total dry weights of 7.7 and 15.1 kJ UV-B-treated plants were 21 and 30% lower than the control plants, respectively. At the final harvest, plants under 720 μmol CO_2 mol^{-1} had a 36% higher total biomass than plants under 360 μmol CO_2 mol^{-1}, averaged across the three UV-B treatments. Total biomass produced was lowered by 12% for the 7.7 kJ UV-B treatment and by 69% for the 15.1 kJ UV-B treatment at 66 DAE, compared to the control (Table 5). Elevated [CO_2] increased fruit dry matter accumulation only in no UV-B control plants because fruit dry weight was similar in the ambient or elevated CO_2 treatments when plants were grown under 7.7 or 15.1 kJ m^{-2} d^{-1} UV-B radiation. The 7.7 kJ UV-B radiation did not affect leaf and stem dry weights, but significantly decreased fruit dry weight compared to the control. The 15.1 kJ UV-B radiation decreased dry matter accumulations of all plant parts, and caused the greatest decrease in fruit weight among plant organs.

Leaf photosynthesis

Net photosynthetic rate (P_n) of the uppermost fully expanded mainstem leaves of control plants was stimulated 44% by elevated [CO_2] (Fig. 2). Leaf P_n of plants grown under 7.7 kJ m^{-2} d^{-1} of UV-B radiation did not differ from that of the control at either CO_2 level at any time of measurements. Averaged across CO_2 treatments, leaf P_n of plants exposed to 15.1 kJ m^{-2} d^{-1} of UV-B radiation was decreased 33% at 28 DAE, 47% at 44 DAE, and 42% at 64 DAE in comparison with the control plants. A significant $CO_2 \times$ UV-B interactive effect was detected in leaf P_n at 44 and 64 DAE. For instance, in comparison with the control plants, the 15.1 kJ UV-B-treated plants had a 38% (44 DAE) or 33% (64 DAE) lower leaf P_n in ambient [CO_2], but 53 or 49% lower leaf P_n under elevated [CO_2].

In general, plants grown in elevated [CO_2] had 11–19% lower stomatal conductance than plants grown in ambient [CO_2] (data not shown). The effect of UV-B radiation on stomatal conductance was similar to the trends observed in leaf P_n. Intercellular [CO_2], on the other hand, did not differ among the UV-B treatments in ambient CO_2 level with a mean of about 260 μmol mol^{-1} (data not shown), but the values of intercellular [CO_2] of 0, 7.7 and 15.1 kJ UV-B-treated plants were 499, 560 and 586 μmol mol^{-1}, respectively, under elevated CO_2 conditions.

Table 4. Effect of elevated CO_2 and UV-B radiation on above-ground dry matter accumulation (g plant^{-1}) in cotton, 14 and 24 d after emergence (DAE)

CO_2 (μmol mol^{-1})	UV-B (kJ m^{-2} d^{-1})	14 DAE			24 DAE		
		Leaves	Stems	Total	Leaves	Stems	Total
360	0.0	0.50 ± 0.03 b*	0.15 ± 0.01 c	0.65 ± 0.04 b	2.27 ± 0.24 b	1.40 ± 0.10 b	3.67 ± 0.35 b
	7.7	0.49 ± 0.02 bc	0.15 ± 0.01 c	0.64 ± 0.03 bc	1.90 ± 0.14 bc	1.11 ± 0.08 c	3.01 ± 0.22 c
	15.1	0.40 ± 0.03 c	0.12 ± 0.01 c	0.52 ± 0.04 c	1.52 ± 0.10 c	0.82 ± 0.06 d	2.34 ± 0.15 d
720	0.0	0.70 ± 0.03 a	0.23 ± 0.01 ab	0.93 ± 0.04 a	2.91 ± 0.26 a	1.92 ± 0.14 a	4.83 ± 0.39 a
	7.7	0.65 ± 0.03 a	0.20 ± 0.01 b	0.85 ± 0.04 a	2.32 ± 0.25 b	1.42 ± 0.15 b	3.72 ± 0.40 b
	15.1	0.66 ± 0.04 a	0.24 ± 0.02 a	0.90 ± 0.06 a	2.30 ± 0.16 b	1.33 ± 0.07 bc	3.63 ± 0.23 b
P (CO_2)		< 0.0001	< 0.0001	< 0.0001	0.0003	< 0.0001	0.0002
P (UV-B)		0.0789	0.2948	0.1427	0.0025	< 0.0001	0.0023
P ($CO_2 \times$ UV-B)		0.3110	0.0443	0.2874	0.6443	0.5071	0.5561

Each value is the mean ± SE of nine plants.
*Means followed by the same letter within a column are not significantly different ($P > 0.05$).

Table 5. Effect of elevated CO_2 and UV-B radiation on dry matter accumulation (g plant^{-1}) of leaves, fruits (bolls + flowers + floral buds), stems, and roots at the final harvest, 66 d after emergence. Each value is the mean ± SE of nine plants

CO_2 (μmol mol^{-1})	UV-B (kJ m^{-2} d^{-1})	Plant parts				
		Leaves	Fruits	Stems	Roots	Total
360	0.0	38.7 ± 4.0 b*	20.3 ± 2.3 b	50.7 ± 4.7 c	9.8	119.6 ± 10.3 c
	7.7	36.4 ± 1.8 b	16.4 ± 1.5 c	52.0 ± 2.4 c	8.2	113.0 ± 5.1 c
	15.1	16.2 ± 1.3 c	3.0 ± 0.9 d	14.7 ± 1.8 e	3.2	37.1 ± 3.9 d
720	0.0	54.0 ± 2.8 a	26.1 ± 2.0 a	78.9 ± 3.2 a	12.1	171.1 ± 7.1 a
	7.7	48.4 ± 2.8 a	15.1 ± 1.6 c	66.8 ± 3.6 b	12.6	142.9 ± 7.3 b
	15.1	19.9 ± 1.6 c	2.2 ± 0.5 d	27.2 ± 2.3 d	4.1	53.4 ± 4.2 d
P (CO_2)		< 0.0001	0.6934	< 0.0001	– †	< 0.0001
P (UV-B)		< 0.0001	< 0.0001	< 0.0001	–	< 0.0001
P (CO_2 × UV-B)		0.0695	0.0345	0. 1863	–	0.0364

*Means followed by the same letter within a column are not significantly different ($P > 0.05$).
†Statistical analysis was not carried out because roots were harvested from entire soil bin and not for each plant within each SPAR unit.

Leaf pigments

Elevated [CO_2] did not affect leaf total chlorophyll, carotenoid concentrations or the chlorophyll a : b ratio (Fig. 3). There were no interactive effects on the pigment components between CO_2 and UV-B radiation treatments. Total chlorophyll concentration decreased and chlorophyll a : b ratio increased with the increase in UV-B radiation. Averaged across the two CO_2 levels, chlorophyll concentrations of 0, 7.7 and 15.1 kJ UV-B-treated plants were 402, 368 and 326 mg m^{-2}, respectively; chlorophyll a : b ratios were 4.09, 4.38 and 4.47, respectively. Carotenoid concentration did not differ between the control and 7.7 kJ UV-B-treated plants, but was 16% lower in plants exposed to 15.1 kJ m^{-2} d^{-1} UV-B radiation.

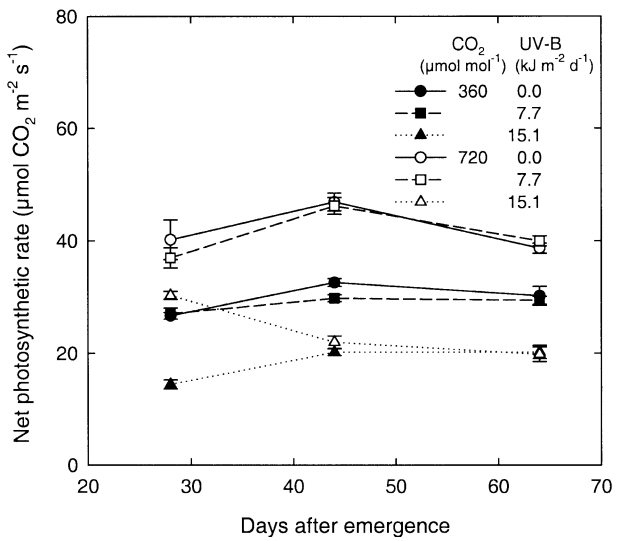

Figure 2. Effects of elevated CO_2 and UV-B radiation on net photosynthetic rate of uppermost, fully expanded mainstem leaves at 28, 44 and 64 d after emergence. Each data point is the mean ± SE of five plants.

Leaf non-structural carbohydrates

Because leaf non-structural carbohydrate concentrations did not differ statistically between the two measurement dates, the data were averaged across sampling dates (Fig. 4). Elevated [CO_2] significantly increased leaf glucose ($P < 0.05$), sucrose ($P < 0.01$), and starch ($P < 0.0001$) concentrations, but did not affect fructose concentration. As a result, plants grown in elevated [CO_2] had 118% higher total non-structural carbohydrates, 28% higher glucose and sucrose, and 191% more starch than plants grown in ambient [CO_2]. No statistical differences in the concentrations of non-structural carbohydrates were detected among the UV-B treatments at ambient [CO_2]. Under elevated CO_2 condition, however, leaf sucrose and starch decreased as UV-B radiation increased; the 7.7 kJ m^{-2} d^{-1} UV-B radiation did not affect leaf glucose and fructose, but the 15.1 kJ m^{-2} d^{-1} UV-B radiation decreased the concentration of both sugars compared to the controls or 7.7 kJ UV-B-treated plants.

DISCUSSION

Effects of elevated CO_2

The results of elevated [CO_2] stimulating growth and dry matter accumulation of cotton plants grown under no UV-B and 7.7 kJ m^{-2} d^{-1} UV-B conditions are similar to earlier reports (Mauney *et al.* 1994; Reddy *et al.* 2000). Elevated [CO_2] affected neither cotton leaf chlorophyll concentrations nor chlorophyll a : b ratio in the present study (Fig. 3). This is in agreement with the findings in wheat by Monje & Bugbee (1998), but inconsistent with Radoglou & Jarvis (1992), who reported elevated CO_2 decreased leaf chlorophyll concentration of *Phaseolus vulgaris*. Numerous studies have shown that elevated [CO_2] increases P_n in C_3 plants because higher [CO_2] can suppress RuBP oxygenase activity; decrease photorespiration and increase carbon assimilates for plant growth and development (Lawlor & Mitchell 2000). Our results also indicate that elevated [CO_2] signifi-

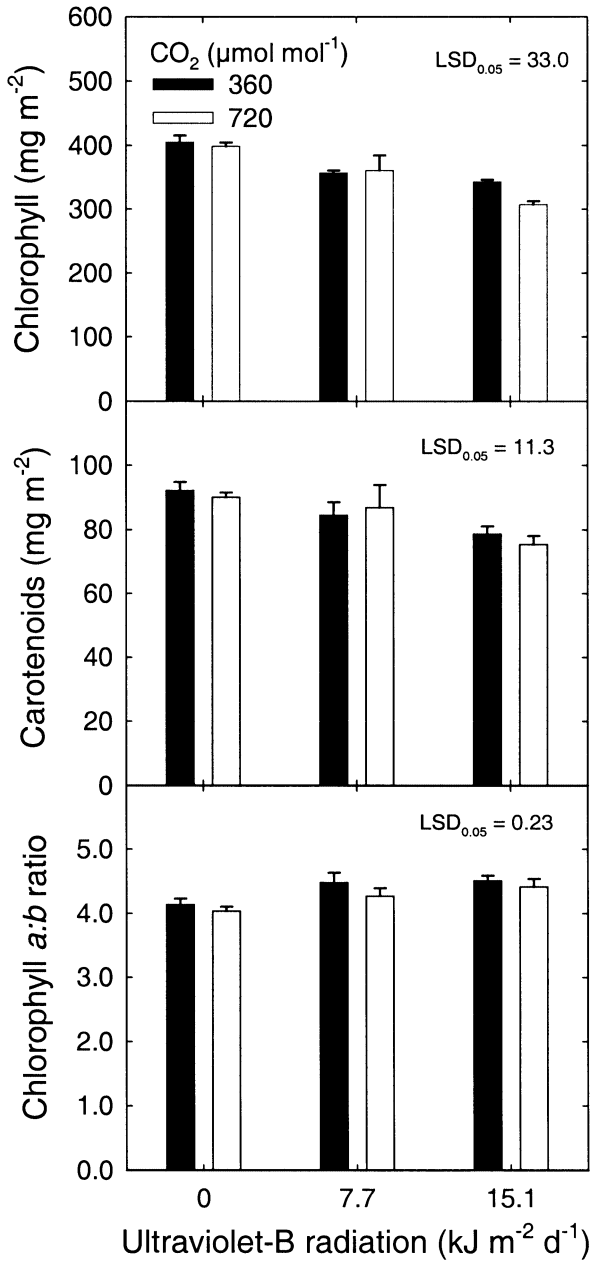

Figure 3. Effects of elevated CO_2 and UV-B radiation on concentrations of leaf chlorophyll and carotenoids, and chlorophyll $a:b$ ratio. Data represent means + 1 SE of six leaf samples measured at first flower and 3 weeks after the first flower.

cantly enhanced cotton leaf P_n under either no UV-B or 7.7 kJ m^{-2} d^{-1} UV-B radiation.

Leaf non-structural carbohydrate concentrations are closely related to leaf P_n. Our results of elevated [CO_2] increasing cotton leaf non-structural carbohydrate (especially, sucrose and starch) concentrations are similar to the findings in faba bean (Visser *et al*. 1997) and in *Ricinus communis* (Grimmer, Bachfischer & Komor 1999). Sucrose controls the expression of ADP-glucose pyrophosphorylase, leading to a shift of carbohydrate partitioning into starch when sucrose synthesis is more rapid than consumption and/or export at elevated [CO_2] (Grimmer *et al*. 1999). Although leaf photosynthetic acclimation may exist when plants are grown under elevated [CO_2] (Visser *et al*. 1997) due to accumulation of non-structural carbohydrates in leaves, our results clearly indicated that elevated [CO_2] stimulated leaf P_n (Fig. 2) and increased photo-assimilate supply, resulting in rapid growth and biomass accumulation in cotton (Tables 4 & 5). These results are consistent with the findings of Begonia *et al*. (1996).

Effects of UV-B radiation

Plant growth, physiological, and yield responses to UV-B radiation differ considerably among crop species or cultivars (Corlett *et al*. 1997; Krupa *et al*. 1998). Our study revealed that UV-B radiation of 7.7 kJ m^{-2} d^{-1}, the amount essentially equivalent to that found on summer clear days in today's ambient environment in the study site (TOMS 2002), did not affect cotton growth and development, but higher levels of UV-B radiation (15.1 kJ m^{-2} d^{-1}), the amount expected to occur with a 30% depletion in the stratospheric ozone, significantly reduced stem elongation rate, leaf area and dry matter accumulation. Decreased plant height due to high UV-B radiation was closely related to shorter internodes rather than a fewer number of nodes. The results of UV-B radiation effect on cotton growth in our study agree with the earlier reports in pea (Nogues *et al*. 1998) and many other crops (Krupa *et al*. 1998).

In terms of the components of biomass, UV-B radiation had the greatest effect on fruit dry weight. Although total biomass and leaf P_n of the 7.7 kJ UV-B-treated plants did not differ from that of 0 kJ UV-B-treated control plants under ambient CO_2 level, the fruit dry weight was significantly lower as UV-B levels increased (Table 5). Reduction in fruit dry weight was related to a higher fruit abscission or fewer bolls retained per plant. Furthermore, floral bud abscission was more than boll abscission in both the 7.7 and 15.1 kJ UV-B treatments. The increase in floral bud abscission by 7.7 kJ UV-B radiation was probably associated with some other causes rather than photo-assimilate supply, because either leaf P_n or leaf non-structural carbohydrates were not different between the 7.7 kJ UV-B-treated plants and the control plants. Demchik & Day (1996) demonstrated that enhanced UV-B radiation decreased pollen quantity and quality of *Brassica rapa*. We expect pollen sterility or a reduction in viable pollen production may be one of factors increasing young boll abscission at high UV-B radiation. The mechanisms of UV-B radiation effect on cotton fruit abscission still need further investigation. In a recent study, Song *et al*. (1999) found that a daily supplement of 9.2 kJ m^{-2} UV (280–400 nm) radiation, for 169 d, decreased dry matter production of field-grown cotton. Our study further indicated that decreased total biomass production, due to high UV-B radiation, was closely related to both small leaf area and low leaf P_n during squaring and fruiting (R^2 = 0.73–0.97, Tables 2 & 5; Fig. 2). The result of elevated UV-B causing the greatest decrease in the fruit dry weight among plant parts due to high fruit

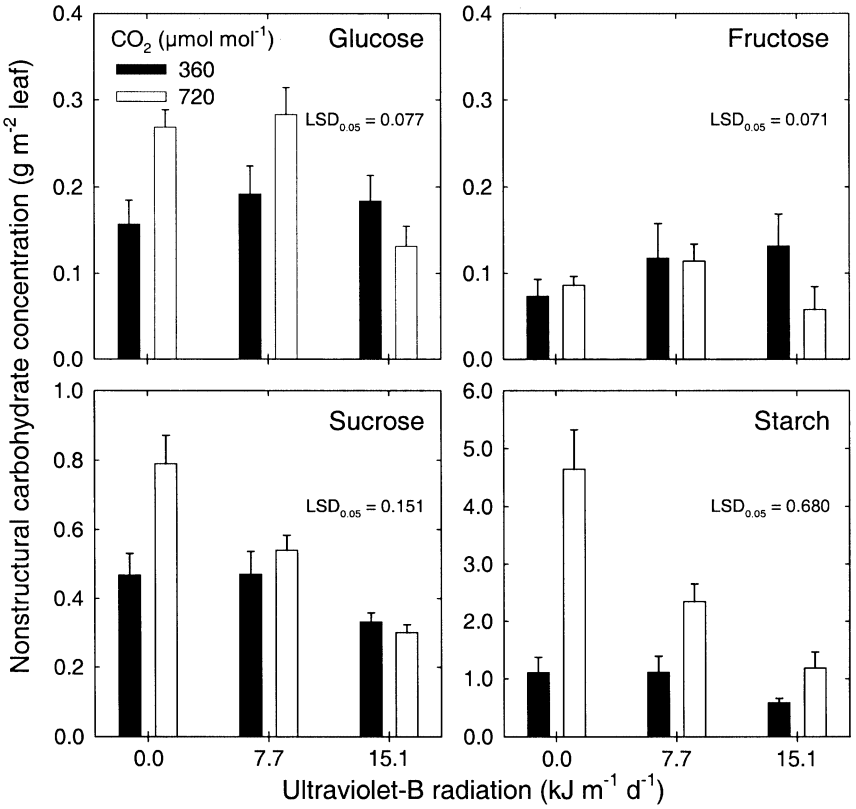

Figure 4. Effects of elevated CO_2 and UV-B radiation on leaf non-structural carbohydrate concentrations. Data represent means + 1 SE of six leaf samples measured at first flower and 3 weeks after the first flower.

abscission in this study is consistent with that in pea (Mepsted *et al*. 1996).

The mechanisms of enhanced UV-B radiation effect on photosynthesis have been reviewed in detail by Allen, Nogues & Baker (1998), including aspects of the photophosphorylation reaction of the thylakoid membrane (i.e. PSII), the CO_2-fixation reactions of the Calvin cycle, and stomatal control of CO_2 supply. The response of leaf P_n to enhanced UV-B radiation depends on crop species, cultivar, environmental condition, UV-B dosage, and the PAR : UV-B ratio. In our study, a daily 15.1 kJ m^{-2} UV-B radiation significantly decreased cotton leaf P_n. Similarly, under field conditions, a daily supplement of 5–11 kJ m^{-2} UV-B decreased leaf P_n of soybean (Sullivan & Teramura 1990) and snapbean (Pal *et al*. 1999). In contrast, Beyschlag *et al*. (1988) reported that enhanced UV-B radiation, equivalent to 20% O_3 depletion, with a modulated delivery system did not affect leaf P_n in wheat under either greenhouse or field conditions. The difference between our results and the findings of Beyschlag *et al*. (1988) was probably associated with crop species, experimental conditions, and UV-B supplementation systems. Our study was carried out under controlled environments with a square-wave UV-B delivery system providing a higher UV-B dosage (15.1 kJ m^{-2} d^{-1}). Although the daily mean PAR was high (18 MJ m^{-2}) in the present study, the square-wave UV-B delivery system usually provides higher UV-B dosages on overcast days (Allen *et al*. 1998; Musil *et al*. 2002). However, the main objective of our study was to determine the potential mechanism of UV-B and CO_2 interactive effects on plant growth and physiology while keeping other growth conditions favourable.

The results of decreased chlorophyll concentration in response to UV-B radiation in this study are consistent with earlier reports in other crops (Li *et al*. 2000; Alexieva *et al*. 2001), but our results of increased chlorophyll *a* : *b* ratio in response to UV-B radiation do not agree with Li *et al*. (2000), who found that UV-B radiation decreased chlorophyll *a* : *b* ratio in wheat. Britz & Adamse (1994) documented that UV-B radiation increased leaf non-structural carbohydrates of cucumber seedlings, resulting in an increase in specific leaf weight. In contrast, UV-B radiation either did not (under ambient [CO_2]) or did (under elevated [CO_2]) decrease cotton leaf non-structural carbohydrate concentrations in our study (Fig. 4). Furthermore, the effects of UV-B radiation on leaf carbohydrates were quite different from those on leaf P_n. Under elevated [CO_2], the leaf P_n of 7.7 kJ UV-B-treated plants did not differ from untreated plants, but the former had a significantly lower total non-structural carbohydrate concentration than the later. It is possible that these plants used photo-assimilates to produce phenolics and other UV-screening compounds when they were exposed to UV-B radiation (Alexieva *et al*. 2001).

Interactive effects of CO_2 and UV-B radiation

Some CO_2 × UV-B interactive effects were detected on certain growth and physiological parameters measured in this study. For instance, when CO_2 levels were increased to 720

from 360 μmol mol^{-1}, cotton leaf P_n increased by about 40% under no UV-B or 7.7 kJ m^{-2} d^{-1} UV-B radiation, whereas leaf P_n was almost unchanged by elevated [CO_2] under 15.1 kJ UV-B radiation during flowering and fruiting (44 and 64 DAE). In an earlier study, Adamse & Britz (1992) found that UV-B-induced reductions in plant growth were less severe when elevated [CO_2] was used to increase P_n. Rozema *et al*. (1997) also reported a significant $CO_2 \times$ UV–B interaction for plant biomass production in *Elymus athericus,* as plant growth was much less reduced by enhanced UV-B radiation at 720 μmol CO_2 mol^{-1} than at ambient atmospheric [CO_2]. However, the evidence of elevated [CO_2] mitigating UV-B detrimental effect on cotton dry matter accumulation was not detected in the present study. The interactive effect of [CO_2] and UV-B radiation on fruit dry weight was detected such that plants grown in elevated [CO_2] appeared to be more sensitive to high UV-B radiation, in comparison with plants grown in ambient CO_2 level (Table 5). Under ambient [CO_2], fruit dry weights of the 7.7 and 15.1 kJ UV-B-treated plants were 81 and 15% of the control plants, respectively. In contrast, fruit dry weights of the two UV-B treatments were only 58 and 11% of the control under elevated [CO_2]. Therefore, an important finding of this study is that elevated [CO_2] did not mitigate the detrimental effects of high UV-B radiation on cotton growth and physiology, especially fruit growth.

A recent study (Smith, Burritt & Bannister 2000) reported that dry matter accumulation is a good indicator of screening plant sensitivity to UV-B radiation among vegetable crop species, and a rapid growth rate renders plants more sensitive to the injurious effects of UV-B radiation. Based on the findings of Smith *et al*. (2000) and our results, we speculate that the sensitivity of cotton to UV-B radiation may change with growth stages and be most sensitive to high UV-B radiation during squaring and early flowering when plants are growing rapidly. Cotton growth is often accelerated by CO_2 enrichment, as shown in Fig. 1 and in Reddy, Hodges & McKinion (1997). Thus, we observed more injurious effects from the UV-B radiation in plants grown in elevated [CO_2], which was supported from measurements of P_n and soluble sugars in the uppermost fully expanded leaves (Figs 2 and 4).

As mentioned earlier, this experiment was conducted in sunlit chambers under controlled environments and using a square-wave UV-B delivery system. The square-wave UV-B system usually overestimates UV-B dosages on overcast days (Musil *et al*. 2002). Data for each treatment were collected from nine plants in a SPAR unit due to limited numbers of chambers. Although evidence indicated no systematic differences among the chambers (Table 1), restriction of sufficient numbers of the chambers for individual treatments and square-wave UV-B system might limit our strong conclusions in this study.

In summary, levels near the current maximum solar UV-B radiation (7.7 kJ m^{-2} d^{-1}) observed in United States mid-south cotton production area on sunny days between May and July did not affect cotton leaf P_n and total biomass production, but significantly increased fruit abscission and decreased fruit dry matter accumulation in comparison with plants that received no UV-B radiation. A doubling of the current UV-B radiation to 15.1 kJ m^{-2} d^{-1} further reduced not only fruit production, but also total biomass. Decreased biomass from high UV-B radiation was closely related to both smaller leaf area and lower leaf P_n. Elevated [CO_2] significantly increased P_n, growth and dry matter accumulation in cotton under no UV-B or 7.7 kJ m^{-2} d^{-1} UV-B conditions. However, elevated atmospheric [CO_2] could not alleviate the detrimental effects of high UV-B radiation on cotton P_n and growth, particularly on reproductive growth.

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